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Human ribosomal protein L7 displays an ER binding property and is involved in ribosome-ER association

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Abstract Human ribosomal protein L7 incorporates an ER-binding characteristic. It is evident from the in vivo ER co-localization of the transiently expressed recombinant L7 in mycophenolic acid treated HeLa cells, the in situ detection of the fluorescent L7 at the ER in digitonin-permeablized HeLa cells, and the expression of a similar K_D value to ribosomes binding to the ER. However, no ER co-localization and a lower K_D was observed if the last 50 amino acid residues at the carboxyl end of L7 were removed, implying that the carboxyl region embodies the ER-binding specificity. Based on the inhibitory effect of an anti L7 antibody during ribosome rebinding to the microsome, we suggest that the L7-ER-binding nature could be one of multiple factors that allow a nascent peptide-less ribosome to remain at the ER.

Keywords: Endoplasm reticulum; Microsome; Ribosome; Biosensor

1. Introduction

Rough endoplasmic reticulum (rough ER) is an essential cellular structure that is involved in the machinery for the extrusion of secretory proteins and transmembrane proteins in eukaryotic cells [1,2]. The structure is a result of a co-translational event in which the nascent peptide chain associated ribosome is recruited by the signal recognition particle (SRP) to its cognate SRP receptor (SR) in a GTP-dependent mode following the insertion of the nascent peptide chain into the ER [3]. This leaves the ribosome bound to the ER. The binding is mediated through an interaction between the ribosome and the Sec61 trimeric complex of the translocon, and is believed to facilitate the safe delivery of the growing polypeptide chain into membrane during the co-translation translocation event [4–6]. In addition, the formation of rough ER can be achieved in vitro with nascent peptide-less ribosomes [7–10]. Both nascent peptide chain-dependent and independent ribosome ER-binding implies that there is an intrinsic affinity between the ribosome and the ER that is strong enough to keep the structure of the rough ER intact, and further suggests that the interaction between the intrinsic components of ribosome and the ER could involve another force that allows the nascent chain-less ribosome to attach to the ER. In this respect, several large subunit proteins [11–15] plus 28S rRNA [16] have been suggested to be located at the exit from the tunnel where they face the translocon onto which the ribosome docks, but finding other intrinsic component(s) of the ribosome that contribute to the stability of the rough ER structure has been difficult. Nevertheless, understanding this process is important to the cellular biology of the rough ER.

In an earlier report using reductive methylation as a probe, several large subunit ribosomal proteins including L7 were found to have membrane binding potential [17]. L7 is also known to have other extra ribosomal activities. It is frequently targeted by autoantibodies in rheumatic autoimmune diseases [18], and in some cases, L7 is highly expressed in colorectal cancer cells but not in the normal cells [19]. The full meaning of these L7-associated extra-ribosomal functions has not been revealed as yet. Recently, the structural parts of L7 that are responsible for its normal ribosome functions including the nuclear targeting, ribosome assembly and translation have been elucidated [20] and the carboxyl region of the protein was detected to have strong membrane binding potential [20], which is consistent with a previously suggested role in ER binding [21,22]. Since L7 has not been included in the list of ribosomal components proposed for the association of the ribosome with the ER [12–16], it is important to discuss what role this potential membrane binding may play. One aspect of this is whether the membrane binding is part of the protein's ribosome function or perhaps, that it involves an extra-ribosome role. Based on this, we attempted here to determine the potential of L7 to bind to the ER. Secondly, we characterized the nature of the carboxyl region with respect to L7-ER-binding in vitro and in situ. Finally, we have adapted an antibody inhibition approach to demonstrate that L7 is important to the formation of the ribosome-ER association in vitro. It is proposed that the binding of the ribosome without a nascent peptide chain to the microsome is mediated by a protein–membrane interaction involving L7.

2. Materials and methods

2.1. Cloning and expression of L7 gene

The genes encoding the fall-length of the flag-tagging human ribosomal protein L7 and the carboxyl truncated mutant L7ΔC50 were obtained from a previous study [20]. These genes were subcloned into the prokaryotic expression pET28a plasmid to obtain recombinant proteins.

Recombinant proteins were purified from *Escherichia coli* BL21-codonPlus (DE3) RIL stain (Stratagene Co.) by the standard procedures of Ni-bead chromatography. In most experiments, the his-tag peptide was excised from the recombinant protein by thrombin treatment.

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2.2. Preparations of ribosomes and puromycin/salt-treated microsome (PK-RM)

The ribosomes were prepared from embryonic kidney 293T culture cells as described previously [23]. PK-RM (stripped high salt-treated microsomes) was prepared from dog's pancreas according to the procedures of Kalies and Hartmann [2].

2.3. Microsome floating assay

A procedure for the in vitro isopycnic flotation of reconstituted microsome [24] was adopted and carried out for this study. Radioactive [^{35}S]-L7 was incubated with PK-RM (one OD_{280} unit) in a total reaction volume of 50 μl of binding buffer containing 5 mM Tris–HCl, pH 7.5; 25 mM KCl; 5 mM $MgCl_2$; and 1 mM DTT for 1 h at 4 °C. The reaction mixture was adjusted to 2.1 M sucrose and then placed at the bottom of a discontinuous sucrose gradient of 0.6 ml each of 1.9, 1.7, 1.5, 1.3, and 1.0 M sucrose in binding buffer in a 5-ml Beckman centrifugation tube. After centrifugation (2.5 h at 4 °C at 50,000 rpm in a Beckman SW55 rotor), the gradient was fractionated into five fractions plus a pellet fraction. An aliquot (0.1 ml) of each fraction was taken and the level of radioactivity determined. [^{35}S]-L7 bound to the PK-RM was expected to be in the 1.3 M sucrose fraction when ribosomes are positively bound. The detection of PK-RM associated protein was carried out by autoradiography after SDS polyacrylamide gel electrophoresis.

The same microsome floating procedure was also applied to the antibody inhibitory experiment. Ribosomes prepared from non-transfected 293T cells were pre-incubated with anti L7 antibody before interacting with the microsome. The percentage of PK-RM bound ribosome was calculated from molarity of rRNA molecules extracted from the floating fraction.

2.4. Localization of fluorescent L7 in digitonin-permeablized HeLa cells

Both recombinant L7 and mutant L7ΔC50 were separately labeled with fluorescent Texas red sulfonyl chloride in the dark for 1 h at 4 °C. The linked protein was separated by Sephadex G25 column in PBS buffer.

The preparation of permeablized HeLa cells was carried out by digitonin-treatment. Briefly, HeLa cells were seeded on a coverslip (15 × 15 mm) 1 day prior to the digitonin-treatment. The cells were then washed with cold BD buffer (50 mM HEPES/KOH, at pH 7.3; 3 mM $MgCl_2$; 110 mM KOAc; 5 mM NaOAc; 2 mM DTT; 1 μg/ml aprotinin; 1 μg/ml pepstatin; and 1 μg/ml leupeptin). Cells were then permeablized for 5 min at 4 °C in 1 ml of 40 μg/ml digitonin (Sigma Chem. Co) and subsequently washed three times with cold BD buffer. After excess fluid was drained, the digitonin-treated cells were treated with the Texas Red-conjugated protein (20 μg/ml of PBS) for 30 min. After removing the excess Texas Red-conjugated protein by washing twice with PBS, the presence of fluorescent Texas Red-conjugated proteins was observed under the excitement of a 596 nm laser beam using a confocal microscopy (Leica TCSNT).

2.5. In situ co-localization of transiently expressed flag-tagging L7 on the ER-membrane after mycophenolic acid (MPA) treatment

Plasmid (pCMV-flag-L7) was transfected into HeLa cells that had been seeded onto a coverslip 1 day prior to the transfection. At 6 h post-transfection, the cells were replenished with 2 ml of fresh medium containing 12 μg of MPA. After another 18 h of incubation, the cells were viewed by a confocal fluorescence microscopy as previously described [20]. Simultaneously, the location of the ER-membrane was identified on the same coverslip by co-staining with an anti calnaxin antibody (Strategene Co.). In a parallel experiment, MPA was removed at the end of incubation, and the cells washed with fresh medium two times, then, 2 mM GTP (final concentration) was added to the cell culture. The effect of supplementation with GTP was observed at time intervals of 15 and 30 min after the administration of GTP.

2.6. Kinetic binding assay using an IAsys biosensor

Binding reactions were carried out in an IAsys resonant mirror biosensor (IAsys new^{+}; Affinity Sensors, Cambridge, UK) at 25 °C using aminosaliane surface cuvettes (Affinity Sensors). Immobilization of the PK-RM to the surface of the cuvette was carried out according to the manufacturer's instructions. Briefly, the surface of the cuvette was equilibrated in 10 mM sodium phosphate buffer pH 7.7. After establishment of a baseline, the phosphate buffer was replaced with 200 μl of 0.56 mg/ml of BS^3 (bis-sulfosuccinimidy1 suberate) (Peiere Chem. Co., USA). The surface was then washed repeatedly with phosphate buffer. The addition of BS^3 and washing were repeated twice. PK-RM at a concentration of 4 OD_{280} units was then added to the cuvette and incubated for 10 min. This was followed with three washes with phosphate buffer. A concentration of 2 mg/ml of BSA was added to block the surface, and then the cuvette was again washed with the phosphate buffer. The entire immobilization was monitored by measuring the changes in optical response expressed in units of an arc s (1 arc s = 1/3600°). Once the response reached a stable level, the cuvette was ready for the binding assay.

The binding assay was carried as follows: the PK-RM-immobilized cuvette was equilibrated with ER-binding buffer (5 mM Tris–HCl, pH 7.5; 25 mM KCl; 5 mM $MgCl_2$ and 1 mM DTT). The reaction was initiated by adding a known concentration of ligates (proteins or ribosomes) dissolved in 100 μl of ER-binding buffer, and then the association reaction was followed over a set time, usually 180 s. The cuvette was then washed twice with the ER-binding buffer, and the dissociation of bound ligates into the bulk ER-binding buffer was followed over time. After the completion of the assay, residual bound ligate were removed by two applications of 200 μl 5 mM HCl, and the immobilized ligand (PK-RM) was thus regenerated. The binding and dissociation events were measured as the arc second response in resonance angle arising as ligate in free solution binds to or dissociates from the immobilized PK-RM. In this study seven or eight concentrations, 25 nM; 50 nM; 75 nM; 100 nM; 150 nM; 200 nM; and 300 nM, were used for each protein tested. For measurement of the affinity of ribosome when binding to immobilized PK-RM, a low concentration series of 0.25 nM; 0.5 nM; 0.75 nM; 1 nM; 1.5 nM; 2 nM; 3 nM; 5 nM; and 10 nM of ribosomes was used because of the ribosome's large molecular mass. The ribosome concentration was determined using the relationship 1 OD_{280} unit of 80S = 21.4 pmol of 80S ribosomes. The association and dissociation rate constants, k_{ass} and k_{diss}, were calculated using non-linear curve-fitting FastFit software (Affinity Sensors, provided with the instrument). The affinity constant, K_D value, was calculated from the ratio of k_{diss}/k_{ass}.

3. Results

3.1. The characteristics of L7 binding to PK-RM

L7 and the mutant L7ΔC50 protein were separately expressed in the *E. coli* strain BL21-codonPlus (DE3), which carries extra copies of the argyl, ilyl and leuyl tRNA genes that specifically recognize the eukaryotic preferred codons. The use of this *E. coli* strain ensured that the host cells made full-length protein. The expressed protein was purified using standard Ni-chromotography. In this study, both radioactive and non-radioactive proteins were prepared in order to carry out the experiments described below.

The binding of radioactive [^{35}S]-L7 and [^{35}S]-L7ΔC50 to the PK-RM was separately analyzed using the microsome floating assay. The results found that the floating PK-RM (1.3 M sucrose) fraction, the bottom fraction (2.2 M) and the pellet carried most of the radioactive material (Fig. 1A). After analyzing each fraction by SDS-containing polyacrylamide gel and autoradiograph, the intact [^{35}S]-L7 was mainly detected in the 1.3 M fraction and the pellet fraction (Fig. 1B). In a parallel experiment, mutant [^{35}S]-L7ΔC50 and ribosomal protein [^{35}S]-L5 failed to be located in the 1.3 M fraction, and remained in the bottom fraction (Fig. 1A), suggesting that both proteins were unable to bind PK-RM.

3.2. Kinetics of L7 protein when binding to the PK-RM

To determine the specificity of L7 binding to PK-RM, a resonant mirror biosensor (IAsys) [25] was used to allow efficient measurement. This type of biosensor has been used in a wide range of research fields including structure/function studies,

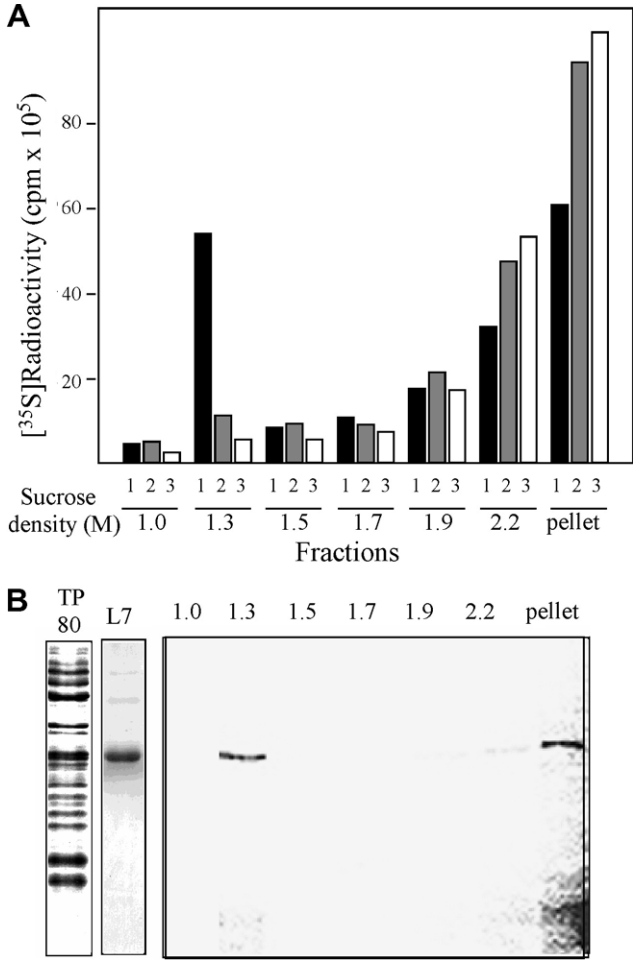

Fig. 1. Binding of L7 to PK-RM. (A) Microsome floating assay for the binding of the ribosomal proteins to PK-RM fractions. The assay was carried out with 40 μg each of [^{35}S]-L7 (1), [^{35}S]-L7ΔC50 (3), or [^{35}S]-L5 (3) proteins and PK-RM in binding buffer as described in the text. All tested radioactive proteins were adjusted to a radio-specificity of 1.5×10^4 cpm/μg by adding corresponding cold recombinant protein for purposes of comparison. (B) The detection of radioactive L7 that is associated with each fraction. The protein in each sucrose fraction was precipitated and analyzed by a SDS polyacrylamide gel electrophoresis. The radioactive protein was visualized by a phosphoimager. TP80 represents total ribosomal proteins from the 80S ribosomes as stained with commassie blue. L7 shows the purity of proteins used in the binding assay.

epitope mapping, kinetic and equilibrium binding analysis, and ligand search [26]. In practice, PK-RM was first immobilized on a pre-coated amino surface of a reactive cuvette. After eliminating unbound material, the modified protein in a binding buffer at various concentrations was reacted with the surface, and the kinetic interaction was measured by the optical shifting. Using this principle, the real-time molecular recognition of L7, mutant L7ΔC50, and ribosome to immobilized PK-RM were obtained (Fig. 2). The affinities of L7, and L7ΔC50, and ribosomes for the PK-RM were listed in Table 1. The binding of L7 to the PK-RM gave a K_D value of 5.03×10^{-7} M, which was close to our previously determined value using the microsome floating assay (4.6×10^{-7} M), whereas, mutant L7ΔC50 gave a much lower K_D of approximated 10^{-5} (Table 1), implying that L7 embodied a ER-binding specificity, and this specificity may mediate through the carboxyl terminal region of the last 50 amino acid residues. In parallel, the binding of ribosome to the PK-RM yielded a higher affinity of 3.04×10^{-8} M (Fig. 2; Table 1) which was comparable to the reported value determined by the conventional microsome floating assay [8], and this was 6-fold higher than that of L7 (Table 1). Nevertheless, this data suggest that in addition to other reported ribosomal component(s) that are engaged in ribosome binding to the ER, L7 may have a role in the association of ribosome and the ER.

3.3. Sub-cellular localization of fluorescent L7 protein in permeablized HeLa cells

To establish if the 'in vitro' observation of L7 binding to PK-RM also exists as a physiological interaction of the L7 protein with the ER-membrane inside living cells, the cellular localization of the L7 protein and mutant L7ΔC50 was examined in permeablized cells. This was carried out by applying Texas red-conjugated L7 (TR-L7) and L7ΔC50 (TR-L7ΔC50) to digitonin-treated HeLa cells and examining them under fluorescent confocal microscopy. The result indicated that TR-L7 was located at the nuclear envelope and at ER-like structures in the cytoplasm (Fig. 3). This contrasted with TR-L7ΔC50, which showed a dispersed appearance through out the cytoplasm (Fig. 3). The in situ observation implied that L7 could have ER properties, and again that such binding might be mediated by the carboxyl region of the protein.

3.4. Sub-cellular localization of transiently expressed flag-tagging L7 protein in a mycophenolic acid (MPA) treated HeLa cell

To re-enforce the in situ observation, the sub-cellular distribution of transiently expressed L7 in HeLa cells was examined. As indicated by our recent report [20], the transiently expressed recombinant L7 was hard to locate in the cytoplasm of the transfected cells because the protein was rapidly transported to nucleus after being synthesized. In order to catch the expressed recombinant L7 in the cytoplasm and to examine whether or not the protein was co-localized with the ER, the L7-transfected cells were treated with mycophenolic acid (MPA). The effect of MPA on cells is well known and results in the sequestration of the GTP-pool of the cell [27]. Such an effect consequently blocks the nuclear transport machinery. As a result, we were able to observe the presence of the transiently expressed L7 in cytoplasm (Fig. 4), and found that the cytosolically restrained L7 co-localized with the ER, which was located by staining with an antibody against the ER marker calnexin (Fig. 4). Interestingly, upon removal of the MPA and supplementation with GTP, the cells resumed nuclear transport, and this was evident as a rapid accumulation of the transiently expressed recombinant L7 within the nucleoli (Fig. 4).

3.5. The role that L7 plays in the ribosome during the formation of rough microsomes

To see whether the ER-binding property ascribed to L7 has a role to play in assisting ribosome binding to the ER-membrane, an antibody inhibitory approach was adopted as part of the floating assay. Essentially, one of criteria to conduct such an antibody inhibition ribosome experiment requires the exposure of L7 on the surface of ribosome particle. Accordingly, an immuno dot blotting assay was carried out, and the result showed that the anti L7 antibody has access

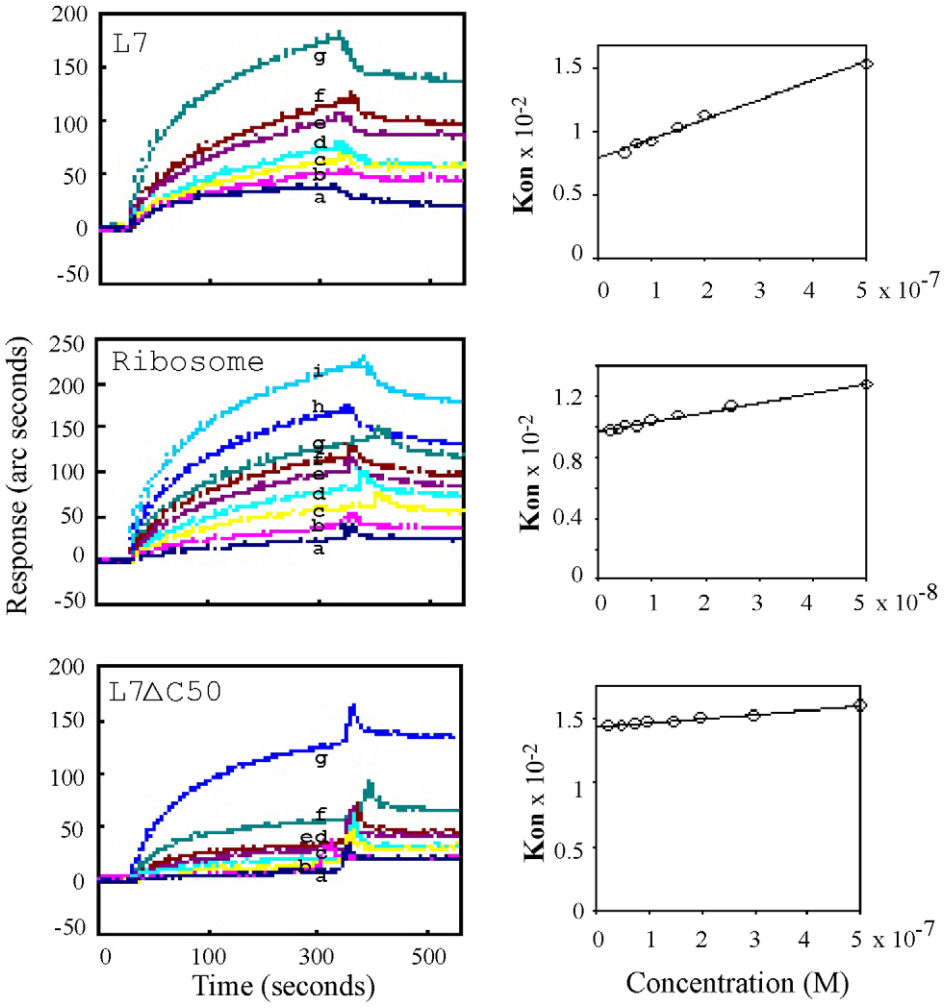

Fig. 2. The binding kinetics of L7 and its mutant L7ΔC50 to PK-RM as determined by a biosensor. On the left, the panels represent the concentration-dependent binding of L7, L7ΔC50, and ribosome to immobilized PK-RM. The concentrations of protein used are: (a) 25 nM; (b) 50 nM; (c) 75 nM; (d), 100 nM; (e) 150 nM; (f) 200 nM; and (g) 500 nM (for L7 and L7ΔC50). A lower concentration series of (a) 0.25 nM; (b) 0.5 nM; (c) 0.75 nM; (d) 1 nM; (e) 1.5 nM; (f) 2 nM; (g) 3 nM; (h) 5 nM; and (i) 10 nM, was used for ribosomes. Two independent sets of binding reactions were performed, of which one is presented. On the right, the panels are the corresponding plots of Kon against ligand (protein or ribosome) concentration and yields a straight line ($r = 0.99$). The slope of this line corresponds to the association rate constant (k_{ass}).The derived K_D values ($K_D = k_{diss}/k_{ass}$) are given in Table 1.

Table 1
Binding kinetics of ribosomal protein L7 and carboxyl deletion mutant to immobilized PK-RM as determined by a biosensor

	k_{ass}[a] (M^{-1} s^{-1})	r[b]	k_{diss}[c] (s^{-1})	K_D[d] (M)
L7	15348	0.99	0.00774	5.03×10^{-7}
L7ΔC50	3339	0.99	0.00084	2.5×10^{-5}
Ribosome	317993	0.99	0.00938	3.04×10^{-8}

[a]The value for k_{ass} are a combination of two independent experiments and are derived from the deviation of the data from a one-site binding model, which was calculated by matrix inversion using the FastFit software provided with the instrument.
[b]The correlation coefficient for the linear regression of the Kon values was used to obtain k_{ass}.
[c]The k_{diss} is the mean = S.E. of at least 7 values, obtained at various concentration of protein.
[d]The K_D was calculated from the ratio of k_{diss}/k_{ass} of the two kinetic parameters.

to and able to interact with ribosomes prepared from 293T cells (Fig. 5B); this confirms that L7 is indeed exposed on the surface of the ribosome. With this confirmation in mind, the ribosomes were pre-incubated with different amount of anti L7 antibodies and then subjected to a microsome floating assay. The result showed that binding of ribosome to ER underwent an approximately 75% decrease if the ribosomes were pre-incubated with an anti L7 antibody (Fig. 5C), whereas, the application of non-immune serum has little effect on ribosome rebinding to the ER (Fig. 5C). It has to be pointed out that increasing amounts of antibody was never able to achieve a complete inhibition of binding (data not shown). Nevertheless, the result of the antibody inhibitory effect clearly suggests that L7 may, perhaps with other molecules, carry out a role in helping the ribosome to associate with the microsome.

4. Discussion

There is overwhelming evidence that the rough ER contains at least two classes of ribosomes, one class is undergoing co-translation translocation in form of the polysome and the other

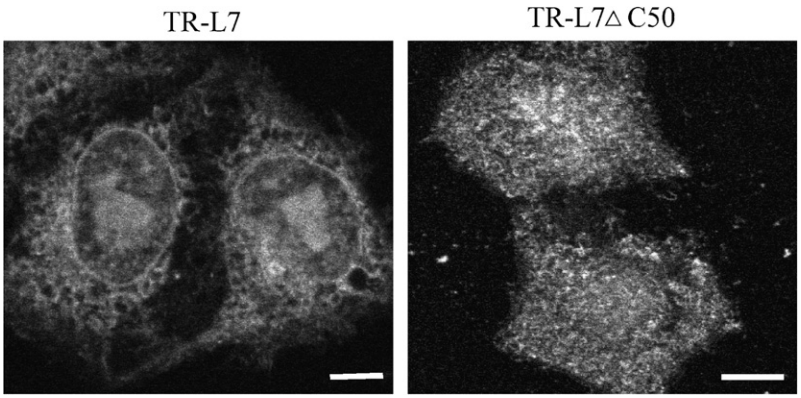

Fig. 3. Sub-cellular distribution of L7 and mutant L7ΔC50 in digitonin permeablized HeLa cells. Fluorescent Texas Red-conjugated L7 and similar L7ΔC50 were separately applied to the permeablized HeLa cells. The location of the Texas Red-L7 (TR-L7), and Texas Red-L7ΔC50 (TR-L7ΔC50) were viewed using a confocal microscopy. Bar = 5 μm.

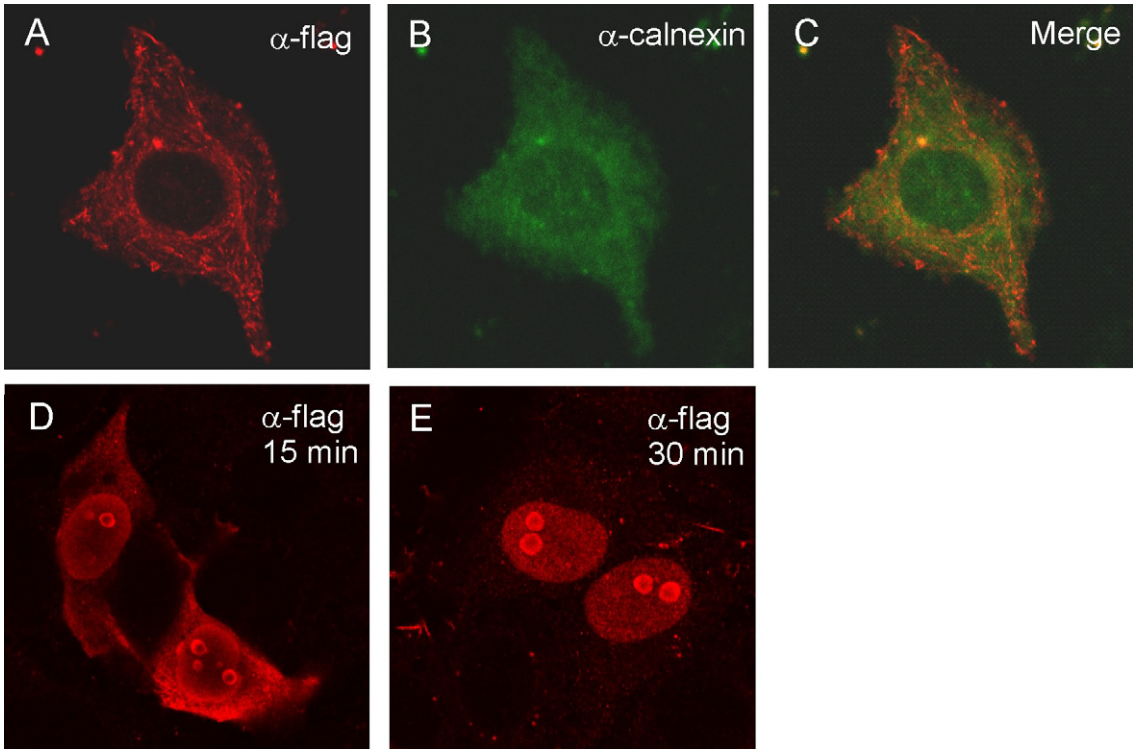

Fig. 4. Sub-cellular localization of the transiently expressed recombinant L7 in MPA-treated HeLa cells. Panel A showed the cellular distribution of the transiently expressed flag-tagged L7 in a post-transfected cell that has been treated with MPA as detected by an anti flag antibody (α-flag). Panel B shows the same cell stained with an anti-calnaxin antibody (α-calnaxin). Panel C is a super-imposed picture A and B (merge) that co-localizes L7 and the ER membrane. The staining of L7 has been assigned in false red color and the location of the ER membrane is shown in false green color. The co-localization of both is shown yellow orange. Panels D and E showed the nucleolar accumulation of fluorescent L7 in a previously MPA-treatment that has had the MPA treatment removed and has been supplemented with GTP. The observations were taken at 15 min and 30 min, after releasing the MPA-treatment and supplementation with GTP. Bar = 5 μm.

class is consist of ribosomes that have finished protein synthesis. On one hand, the mechanism of ER-anchoring for ribosome processing of co-translation translocation is known to be mediated by a protein-protein or a protein-RNA interaction [4,5,10,28], where ribosomes interacted with the translocon of the ER. On the other hand, how the ribosome remains on ER after the translation has been terminated is matter of debate. It has been suggested that at the termination of translation, the large subunit assumed a stable conformation allowing it to stay on the ER with a high affinity for the membrane, thereby allowing continuous membrane association [5,28]. This implies that the two classes of ribosomes might not share the same mechanism with respect to its position on the ER, and that other forces, besides using the translocon [29], are needed that allows the nascent peptide-less ribosome to attach to the ER. Considering that most in vitro microsome floating assays carried out, unless it is specifically mentioned, involved ribosomes without an associated a nascent peptide chain, this suggests that ribosome-associating microsome could mimic the rough ER that is in a situation of translation termination.

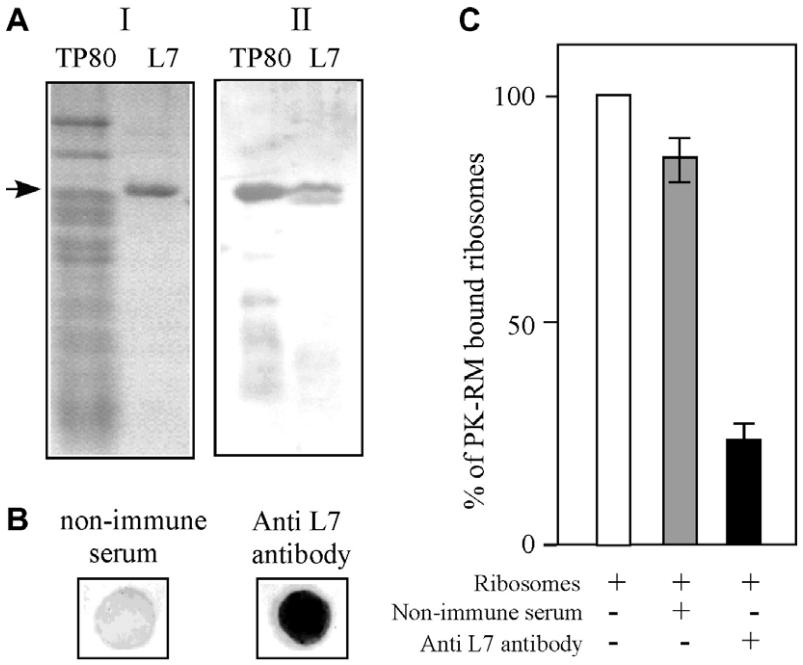

Fig. 5. Inhibitory effect of anti L7 antibody on ribosome binding to the PK-RM. (A) The specificity of anti L7 antibody, to total protein of 80S (TP80) and purity of recombinant L7 protein (L7) were separated by SDS-containing polyacrylamide gel electrophoresis and visualized by a coomassie staining (I); the same separation was analyzed by Western blot using anti L7 antibody (II). Arrow indicated the position of L7. (B) The detection of surface exposure of L7 by the ribosome dot blotting assay. Each dot contains 0.1 OD_{254} unit of ribosomes prepared from non-transfected cells, and was reacted with an anti L7 antibody or the non-immune serum. The detection of reactivity was carried out by the non-radioactive ECL (enhanced chemilluminescence) system with a second antibody. (C) The inhibitory effect of an anti L7 antibody. The floating assay was carried out with ribosomes prepared from non-transfected 293T cells in the present of an anti L7 antibody (access moles presence), or with the non-immune serum. Total rRNA was extracted from each floating fraction and analyzed in agarose gel. The amount of 28S rRNA was measured and used to calculate the percent PK-RM bound ribosomes. The ribosome bound to PK-RM without antibodies was taken as 100% binding.

In this study, we suggest that the large subunit ribosomal protein L7 may be part of the mechanism in nascent peptide chain less ribosome–microsome association because of its ER-membrane binding nature. This was evident from the biochemical data that L7 was capable of binding the ER with an affinity close to that of the ribosome binding to the ER, together with the physical co-localization of the protein with the ER in cells. In addition, ER affinity and ER-localization were no longer observed if the last 50 amino acid residues at the carboxyl end of L7 were removed. This suggests that L7 embodies an ER-binding specificity, and the specificity is implemented by the carboxyl region of protein. The most important link between the L7-ER binding activity and ribosome-ER association comes from the fact that the rebinding of ribosome to the microsome is significantly inhibited by the application of an antibody specifically against L7. This confirms that L7 has a role to plays in assisting the ribosome remaining on the ER membrane.

It is clear from the 2.4 angstrom resolution view of the 50S *H. marismortui* ribosome subunit [11] that the location of L30, the homologous protein to eukaryotic L7, is on the bottom side of the 50S subunit, but that the position is some distance from the exit site of the nascent chain. This is not really a problem, but rather suggests that the mechanism of L7 guided ribosome ER-binding may not involve the translocon. As a matter of fact, our results have shown that the application of anti L7 antibody does not completely inhibit ribosome binding to the ER, and this could be because of some ribosomes are able to use the translocon to attach to the ER. In addition, based on the earlier finding that the carboxyl end of L7 has membrane binding potential [20], it is possible to speculate that the binding of L7 to the microsome might be membrane-mediated. The importance of the carboxyl region of L7 to the ER-binding is further seen in the biochemical kinetic data where L7 alone has a comparable dissociation constant K_D value close to that of the ribosome binding to the ER, whereas, L7ΔC50 has a much lower K_D of 10^{-5}. The determined K_D for L7 could represent the stable force that keeps the translation terminated ribosome on the ER. We therefore speculate that the membrane might be involved in the retention of the translation terminated ribosome on the ER. The question remains as to how ribosomes make the transition from one site to the other site on the ER, and, what molecules are involved in this transition. In the light of recent suggestions on the role that the TRAM (translocation associated membrane) played in the structure of the rough ER [30,31], this postulated mechanism is highly possible and such a proposition is currently under investigation.

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